IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re United States Patent Application of:		**Docket No.:**	**026086-033.210 US**
Applicant:	**David J. Kyle, et al**	**Examiner:**	**V.E. Bertoglio**
Application No.:	**10/532,344**	**Art Unit:**	**1632**
Date Filed:	**September 2, 2005**	**Conf. No.:**	**1584**
Title:	**SHRIMP AND THE PRODUCTION THEREOF**	**Customer No.:**	**24239**

DECLARATION OF DR. DAVID J. KYLE UNDER 35 U.S.C. §1.132 IN U.S. PATENT APPLICATION NO. 10/532,344

Mail Stop Amendment
Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, David J. Kyle hereby declare:

1. THAT I am a named co-inventor of the invention that is described and claimed in U.S. Patent Application No. 10/532,344 filed in the United States Patent and Trademark Office on September 2, 2005 in the names of David J. Kyle and Robert Bullis for "SHRIMP AND THE PRODUCTION THEREOF" (the "Application").

2. THAT the invention of the Application relates to shrimp having a DHA/EPA (docosahexaenoic acid/eicosapentaenoic acid) ratio greater than 1.0 and production of same. DHA is important to nursing mothers, children and adults in general but the level of DHA is usually quite low in shrimp. Further, it is important that the shrimp have higher levels of DHA relative to EPA because higher levels of EPA are associated with the negative effects such as reduced growth and increased bleeding time in humans. Thus, these higher levels of EPA would not be beneficial to a human consuming such shrimp. As such, we determined that there was a need for shrimp that could deliver DHA levels in an amount higher than EPA.

3. THAT the following Methods and Material were used in the testing procedures:

DHA enrichment of shrimp. Twenty-four 9 liter treatment tanks were set up containing 8 shrimp (1-2 g each) per tank. A larger 60 liter tank housing 64 shrimp was also maintained as a control for the treatment groups. Three diets were prepared starting with a basal feed (Zeigler, SI 35, Ziegler Corp, MD), which was ground up an blended with an enriched algal source of DHA (AquaGrow Gold, Advanced BioNutrition, Corp, Columbia, MD) at a ratio of 1:1 (50% AquaGrow Gold), 1:2 (30% AquaGrow Gold), and 1:4 (20 % AquaGrow Gold). Water was added (30-50 ml/100 g) to make pliable dough and the moist dough was passed through a pasta machine to produce pellets which were air dried at 20°C, sprayed with canola oil, and chopped into 3 cm pellets before feeding to the shrimp. Shrimp were fed the test diets at a daily feeding rate of about 5% of body weight (64 animals per test diet). On a weekly basis over 4 weeks, 15 animals were randomly sampled from each test group and three pools of 5 animals each were prepared. All animals had their heads and exoskeletons removed and were deveined before storing the tail muscle at -20°C before processing. All samples were freeze dried and processed for gas chromatographic analysis of total fatty acids using the routine fatty acid methyl ester (FAME) procedure. FAME data were calculated on the basis of total percentage of certain fatty acids (*i.e.*, DHA, EPA and ARA) as well as amounts and ratios.

4. THAT the results, as set forth in Tables 1 and 2, show that after 4 weeks on the treatment diets the DHA content of the shrimp could be significantly improved in an absolute amount, as well as relative to EPA, with a minimal effect on ARA levels. As a result the newly produced highly nutritional shrimp had a DHA/EPA ratio of greater than 1, as described on page 12 of the application. As shown in Table 1, the percent of DHA in the total amount of fatty acid in the tested tail muscle showed a marked increase in the level of DHA with a concurrent reduction in the level of EPA.

Table 1. Impact of inclusion of AquaGrow (AG) in the diet of shrimp on DHA, EPA and ARA content (% of total FAME) of tail muscle (A.) and ratios thereof (B.).

A.	Inclusion of AquaGrow Gold in diet and % amounts of total FAME					**B.**	Inclusion of AquaGrow Gold in diet			
	0%	20%	33%	50%			0%	20%	33%	50%
ARA	4.1	5.5	6.0	6.1		**DHA/EPA**	0.6	1.2	1.2	1.3
EPA	16.7	12.1	12.0	12.3		**DHA/ARA**	2.5	2.6	2.4	2.5
DHA	10.1	14.2	14.6	15.4		**ARA/EPA**	0.2	0.5	0.5	0.5

Further, the ratio of DHA/EPA was also increased beyond the control ratio of 0.6. Results in Table 2 show that the amount of DHA is increased in the shrimp tissue relative to the amount of EPA.

Table 2. Impact of inclusion of AquaGrow in the diet of shrimp on absolute content of DHA, EPA and ARA (mg Fatty Acid (FA)/g tail muscle tissue) (A.) and ratios thereof (B.).

A.	Inclusion of AquaGrow Gold in diet (mgFA/g tail muscle tissue)					**B.**	Inclusion of AquaGrow Gold in diet			
	0%	20%	33%	50%			0%	20%	33%	50%
ARA	0.3	1.0	0.7	0.8		**DHA/EPA**	0.6	1.1	1.2	1.25
EPA	1.3	2.3	1.3	1.6		**DHA/ARA**	2.4	4.0	3.9	3.7
DHA	0.8	2.7	1.6	2.0		**ARA/EPA**	0.2	0.3	0.3	0.3

Results in Table 3 indicate that this result could be obtained in as little as 1 week of enrichment even at the lower dose of 20% inclusion in the feed.

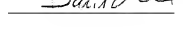
Table 3. Impact of inclusion of AquaGrow (AG) at a level of 20% in the diet of shrimp for only 1 week on percentage and absolute levels of DHA, EPA and ARA in the shrimp tail muscle (A.) and on the ratios calculated based on either percentages or absolute amounts (B.).

A. AG inclusion	% FAME		mg FA/g tissue		**B.** AG inclusion	% FAME		mg FA/g tissue	
	0%	20%	0%	20%		0%	20%	0%	20%
ARA	4.1	3.6	0.3	0.5	**DHA/EPA**	0.6	1.3	0.5	1.3
EPA	16.7	11.2	1.3	1.6	**DHA/ARA**	2.5	3.9	1.6	4.0
DHA	10.1	14.0	0.8	2.1	**ARA/EPA**	0.2	0.3	2.1	0.3

5. THAT viewing the above-data in Table 3, it is evident that the amount of DHA in the tested tissue went from 0.8 mg/g of tissue to 2.1 mg/g of tissue which provided for a DHA/EPA ratio in the tested muscle tissue of 1.3 in only one week.

6. THAT in conclusion, we have found a method to increase levels of DHA without the negative side effects of increased levels of EPA in shrimp tissue.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statement may jeopardize the validity of the application or any patent issued thereon.



David J. Kyle, Ph.D.